Cell. Mol. Biol. 57 (1): 93-99
Published on February 12, 2011; DOI 10.1170/T907

EFFECT OF DIFFERENT DOSES OF SODIUM FLUORIDE ON VARIOUS HYDROXYPROLINE FRACTIONS IN RAT SERUM

N. J. SIDDIQI*, E. A. Al-OMIREENI AND A. S. ALHOMIDA

Department of Biochemistry, College of Science, PO Box 22452, King Saud University, Riyadh – 11495, Saudi Arabia.

Abstract

Human beings are exposed to fluoride through its occurrence in the environment and its presence in various products. The present study was carried out to study the effect of acute doses of sodium fluoride on the body collagen in rats. To evaluate this effect the concentration of collagen breakdown products like different hydroxyproline fractions were determined in serum following the exposure of rats to various concentrations of sodium fluoride. 5 and 10 mg/kg weight dose of NaF caused no significant change in total hydroxyproline but caused significant changes in some of the hydroxyproline fractions. However higher doses of NaF viz., 20 and 30 mg/kg body weight caused significant changes in different hydroxyproline fractions and also a significant decrease in total hydroxyproline indicating the probability of collagen formation in some tissues.

Key words: Hydroxyproline, collagen, rats, serum, sodium fluoride.

Article information
Received on December 17, 2010
Accepted on January 5, 2011

Corresponding author
Dr. N. J. Siddiqi,
Department of Biochemistry, College of Science, PO Box 22452, King Saud University, Riyadh – 11495, Saudi Arabia.
Fax: +966-1-4769137
E-mail: nikhat@ksu.edu.sa

Abbreviations: Hyp : Hydroxyproline.

INTRODUCTION

Fluorine occurs in environment in combination with other elements as a fluoride compounds. Human beings are exposed to fluoride through food (8, 26), drinking water (30) and inhalation (10). Frequent absorption of the fluoride causes tooth decay (18), damage of kidneys (11), bones (4), nerves (22) and muscles (7). The adverse toxic effects of fluoride arise due to a) enzyme inhibition b) collagen break down c) gastric damage and d) disruption of the immune system (2).

Collagen represents the chief structural protein accounting for approximately 30% of all vertebrate body protein. More than 90% of the extracellular protein in tendons, bone and more than 50% in the skin consists of collagen. Connective tissue derives its prominent features such as mechanical strength from collagen. Since most of the scaffolding spectrum in mammals is composed of collagen, different collagen types confer distinct biological properties to various connective tissues in the body (9). The hydroxyproline (Hyp) is a post translational product of proline hydroxylation

catalyzed by the enzyme prolylhydroxylase (19). The occurrence of this amino acid is thought to be confined exclusively to collagen, where it is present in the Y position of the Gly-X-Y repeating tripeptide (17). Consequently, the presence of hydroxyproline in tissues or serum can be used as a measure of collagen or of degradation products of collagen respectively (25). The aim of the present investigation was to study the effect of different doses of sodium fluoride on the Hyp fractions in rat serum which would reflect the overall collagen status of the body.

MATERIALS AND METHODS

Chemicals

Chloramine-T, p-dimethylaminobenzaldeyde (Ehrlich's reagent), L-hydroxyproline, sodium acetate, citric acid, perchloric acid, n-propanol, sodium hydroxide, and acetic acid were purchased from Sigma Chemical Company, St Louis, MO, USA. Double distilled water was used throughout the study.

Animals

Healthy adult male Wistar rats weighting 150-200 g were obtained from breeding laboratory, King Saud University, Riyadh, Saudi Arabia. The animals were labeled by identifying ear notches, housed in clean cages, and placed in the animal care room.

Dose-response of Sodium fluoride

Following one-week acclimatization period, rats were randomly divided into different groups (4-6 rats/group) and individually housed in stainless-steel metabolic cages (Mini Mitter Co., Inc., Bend, Oregon, USA) to allow for the separate collection of feces and urine. Rats were allowed free access to food (Purina rodent chow) and tap water for one day. Baseline values for body weight, food and water intake were obtained during a 24-hour period running from 09:00 to 09:00 h the following morning. After one day control, rats were divided into different groups. The following groups were studied: (i) control rats (n= 4 – 6 rats); (ii) rats injected intraperitonally (ip) normal saline solution (placebo group n = 4 -6 rats); (iii) rats were divided into four subgroups according to the dose of NaF. Rats were injected with a single intra peritoneal doses of (a) 5, (b) 10, (c) 20 and (d) 30 mg NaF /kg body weight /24 hours (NaF treated group, n = 4-6 rats).

Sample preparation

Whole blood was collected without any anticoagulant, transported to the laboratory at 4^0 C where it was allowed to clot at 37^0 C for 30 - 60 minutes and serum was collected by centrifugation at 3000 rpm for 10 minutes. The supernatant thus obtained (serum) was used for biochemical analysis and Hyp determination.

Extraction of Free, Peptide- and Protein-bound Hydroxyproline

Free and protein-bound Hyp was extracted by the method of Varghese et al., 1981 (28) with slight modification. 0.5 mL of the serum was treated with 3 X 2 mL portion of re-rectified absolute alcohol and centrifuged at 3000 rpm for 10 min. The supernatants were pooled and evaporated to dryness. The residue was dissolved in 0.5 mL of distilled water (fraction I) and 50 µl of the extract was used for estimation of free Hyp. The peptide-bound Hyp was determined after alkaline hydrolysis of the ethanol extractable fraction (fraction II). The pellets of the samples were dissolved in 500 µL of distilled water (fraction III) and 50 µL of the extract was used for determination of protein-bound Hyp.

Determination of Hydroxyproline Concentration

Hydroxyproline was measured by the modified alkaline hydrolysis method of Reddy and Enwemeka, 1996 (21). Briefly 50 µL of the sample was added into sodium hydroxide (2 N final concentration). The mixture, was then hydrolyzed by heating in a boiling water bath for about 3-4 h. 900 µL of 56 mM chloramine T reagent was added to the hydrolyzed sample and oxidation was allowed to proceed at room temperature for 25 min. Then 1000 µL of 1 M Ehrlich s reagent (p-dimethylaminobenzaldehyde) was added to the oxidized sample and the chromophore was developed by incubating the samples at 65° C for 20 min. The absorbance was read at 550 nm using Ultrospec 2000 UV/visible Spectrophotometer (Pharmacia Biotech Ltd, Science Park, Cambridge, England). The Hyp concentration in the samples was calculated from the standard curve of Hyp. Further details about the optimization, linearity, specificity, precision and reproducibility of the method has been described previously (23). Serum alkaline phosphatase was determined using automatic autoanalyzer.

RESULTS

Table 1 shows the effect of different doses of NaF on some of serum biochemical parameters in rats. Serum urea nitrogen and protein concentration were found to increase significantly by 10 mg/kg body weight of NaF. However 30 mg/kg body weight of NaF caused a significant decrease in the levels of blood urea nitrogen and protein concentration in serum when compared to control rats.

Table 2 shows the effect of different doses of NaF on some of serum electrolyte concentration in rats. Among the electrolytes there was a significant decrease in serum calcium and phosphorous at 20 and 30 mg/kg body weight of NaF when compared to control group of rats.

Table 3 shows the effect of different doses of NaF on different Hyp fractions in rat serum. NaF doses of 5 and 10 mg/kg caused no significant change in total serum Hyp concentration in rats. However the same doses caused significant changes in different Hyp fractions in the serum. NaF at dose of 5 mg/kg body weight caused an increase in peptide –

Table 1. Effect of Different Doses of Sodium Fluoride on Serum Biochemical Parameters in Rats

Biochemical Parameters	Control	Placebo	5 mg/kg body weight	10 mg/kg body weight	20 mg/kg body weight	30 mg/kg body weight
Urea (mg/dl)	33.5 ± 3.12	34.50 ± 3.32^{ns}	40.75 ± 4.92^{ns}	62.0 ± 9.31***	57.0 ± 8.83***	30.50 ± 4.44*
BUN (mg/dl)	17.75 ± 3.30	17.25 ± 2.22^{ns}	19.75 ± 2.36^{ns}	17.25 ± 1.5*	30.00 ± 6.05**	23.75 ± 6.19^{ns}
Creatinine (mg/dl)	0.37 ± 0.07	0.40 ± 0.06^{ns}	0.55 ± 0.05*	0.36 ± 0.06^{ns}	0.51 ± 0.03^{ns}	0.47 ± 0.09^{ns}
Uric acid (mg/dl)	1.33 ± 0.21	1.28 ± 0.52^{ns}	1.40 ± 0.27^{ns}	1.63 ± 0.15^{ns}	0.70 ± 0.14^{ns}	1.50 ± 0.52^{ns}
Albumin	3.56 ± 0.45	3.71 ± 0.33^{ns}	3.54 ± 4.50^{ns}	3.95 ± 0.17^{ns}	3.10 ± 0.34^{ns}	2.66 ± 0.21^{ns}
Protein	6.20 ± 0.45	6.44 ± 0.43^{ns}	7.35 ± 0.38^{ns}	7.63 ± 0.60*	5.55 ± 0.77^{ns}	5.02 ± 0.59*

Animals were injected with sodium fluoride through intraperitoneal route. Each group consisted of 4 – 6 animals. Rats were sacrificed 24 hours after the treatment.
^{ns} non significant as compared to control group (Tukey's multiple comparision test)
*P < 0.05 compared to control group (Tukey's multiple comparision test)
**P < 0.01 compared to control group (Tukey's multiple comparision test)
***P < 0.001 compared to control group (Tukey's multiple comparision test).

Table 2: Effect of Sodium Fluoride Treatment on Changes in Serum Electrolytes Concentration in Rats

Serum Electrolytes	Control	Placebo	5 mg/kg body weight	10 mg/kg body weight	20 mg/kg body weight	30 mg/kg body weight
Sodium (mEq/I)	143.8 ± 4.35	144.5 ± 6.46^{ns}	148.8 ± 6.5^{ns}	142.8 ± 3.1^{ns}	139.5 ± 2.52^{ns}	136.0 ± 4.24^{ns}
Potassium (mEq/I)	5.58 ± 0.96	6.50 ± 3.48^{ns}	6.20 ± 0.51^{ns}	11.75 ± 0.87***	4.63 ± 0.17^{ns}	4.68 ± 0.50^{ns}
Calcium (mg/dl)	12.34 ± 0.66	12.82 ± 0.23^{ns}	12.75 ± 1.20^{ns}	11.99 ± 0.25^{ns}	9.49 ± 0.13***	8.55 ± 0.19***
Magnesium (mg/dl)	2.45 ± 0.04	2.54 ± 0.32^{ns}	3.21 ± 0.47*	2.44 ± 0.22^{ns}	2.44 ± 0.12^{ns}	2.34 ± 0.45^{ns}
Phosphorous (mg/dl)	10.24 ± 1.64	10.24 ± 2.23^{ns}	12.42 ± 2.43^{ns}	10.80 ± 0.86^{ns}	6.42 ± 0.61*	6.79 ± 0.94**

Animals were injected with sodium fluoride through intraperitoneal route. Each group consisted of 4 – 6 animals. Rats were sacrificed 24 hours after the treatment.
^{ns} non significant as compared to control group (Tukey's multiple comparision test)
*P < 0.05 compared to control group (Tukey's multiple comparision test)
**P < 0.01 compared to control group (Tukey's multiple comparision test)
***P < 0.001 compared to control group (Tukey's multiple comparision test).

Table 3. Effect of different doses of sodium chloride on serum hydroxyproline fractions in rats

Experimental Groups	Free hydroxyproline (µg/ml)	Peptide –bound hydroxyproline (µg/ml)	Protein – bound hydroxyproline (µg/ml)	Total hydroxyproline (µg/ml)
Control	9.00 ± 0.46	71.06 ± 7.0	405.7 ± 56.42	487.9 ± 62.73
Placebo	8.57 ± 0.88^{ns}	72.06 ± 5.8^{ns}	411.9 ± 18.22^{ns}	492.7 ± 22.06^{ns}
5 mg/kg body weight	6.74 ± 2.23^{ns}	173.5 ± 9.10***	254.3 ± 22.53***	434.4 ± 20.48^{ns}
10 mg/kg body weight	12.60 ± 0.41**	153.7 ± 7.57**	270.4 ± 32.63***	436.7 ± 28.55^{ns}
20 mg/kg body weight	3.85 ± 0.31**	125.9 ± 34.68^{ns}	154.9 ± 14.36***	284.6 ± 43.45***
30 mg/kg body weight	4.91 ± 0.67**	219.3 ± 72.22***	138.3 ± 13.99***	362.5 ± 76.74**

Values are expressed as mean ± SD kidney weight (4- 6 rats/group)
^{ns} non significant as compared to control group (Tukey's multiple comparision test);
**P < 0.01 compared to control group (Tukey's multiple comparision test).
***P < 0.001 compared to control group (Tukey's multiple comparision test).

bound Hyp by 144 % ($p < 0.001$) but a decrease in protein bound Hyp by 37% ($p < 0.001$) when compared to control rats. 10 mg/kg body weight dose of NaF caused a significant increase of 40 % and 116 % ($p < 0.01$) in free and peptide- bound Hyp but a decrease in protein- bound Hyp by 33% ($p < 0.001$) when compared to control rats. 20 mg NaF/kg body weight dose caused a decrease in free and protein bound Hyp by 57 and 62% ($p < 0.01$) respectively when compared to control rats. 30 mg/kg body weight NaF caused decrease in free and protein bound Hyp by 45 and 66 % ($p < 0.01$) respectively but an increase in peptide bound Hyp by 209 % ($p < 0.001$) when compared to control rats. Doses of NaF 20 and 30 mg/kg body weight caused a significant decrease ($p < 0.001$) in total Hyp by 42 and 26% respectively when compared to control group.

Figure 1 shows the effect of different doses of NaF on some of serum alkaline phosphatase levels in rats. NaF doses of 20 and 30 mg/kg body weight caused a significant decrease in serum alkaline phosphatase ($P < 0.05$) when compared to control rats.

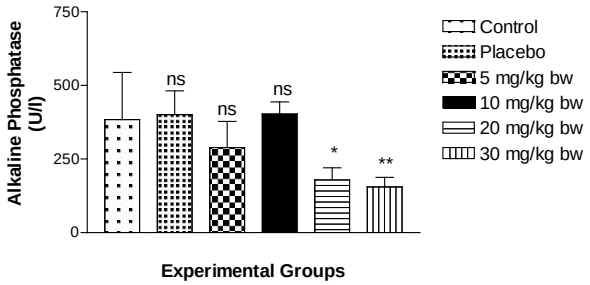

Figure 1. Effect of different doses of sodium fluoride treatment on serum alkaline phosphatase in rats. Animals were injected with sodium fluoride through intraperitoneal route. Each group consisted of 4 – 6 animals. Rats were sacrificed 24 hours after the treatment. ^{ns} non significant as compared to control group (Tukey's multiple comparision test) bw – body weight. *P < 0.05 compared to control group (Tukey's multiple comparision test) **P < 0.01 compared to control group (Tukey's multiple comparision test) ***P < 0.001 compared to control group (Tukey's multiple comparision test).

DISCUSSION

Fluoride is useful in preventing dental caries but excessive intake of fluoride can be toxic. The first noticeable signs of excessive exposure to fluoride is discolouration of the enamel. Abnormalities in mineralization processes affect by and large the osteoarticular system and are associated with changes in the density and structure of the bone presenting as irregular mineralization of the osteoid. Fluorine compounds also act on the organic part of supporting tissues, including collagen and other proteins, and on cells of the connective tissue. These interactions reduce the content of collagen proteins, modify the structure and regularity of collagen fibers and induce mineralization of collagen (13).

Among the biochemical parameters studied in the serum, significant changes were observed in serum urea and protein. There was a significant increase in serum urea concentration with increasing dose of NaF up to 10 mg/kg body weight followed by decrease at 20 and 30 mg/kg body weight. These results together with a decrease in the protein content of the kidneys on NaF treatment (3) suggest that there may be an increased breakdown of protein in the tissues of NaF treated rats. Bouazia et al., 2006, (6) have also demonstrated a decrease in tissue protein levels after sodium fluoride treatment in rats. The increased protein degradation could lead to increase in amino acid content in the body. Since amino acids cannot be stored they could be catabolized by transamination and oxidative deamination. These catabolic reactions could be the source of ammonia which could be used in the synthesis of urea (5). Earlier studies by Birkner et al., 2000 (5) have also shown an increase in serum urea after an acute dose of NaF in rats sacrificed 90 minutes after NaF injection. The decline in serum urea after 30 mg/kg body weight dose of NaF could be due to in inhibition of protein synthesis by NaF. There was also a decline in total protein in serum by higher doses of NaF. These results are in agreement with the studies of Qujeq et al., 2002 (20). Sodium fluoride was found to decrease the calcium concentration in the serum. This may be due to decrease in intestinal absorption of calcium by fluorine (29). Studies by Xin et al., 2006 (29) have shown a decrease in serum calcium and magnesium concentration in pigs treated with an acute dose of NaF. In the present study however there was no significant change in serum magnesium concentration. This may be due to difference in species of experimental animals. Only a 10 mg/kg body weight dose of NaF caused a significant increase in serum potassium. However other doses of NaF caused no significant change in serum potassium

concentration the reason for which is unclear. Earlier studies of Mansour et al., 1981 (15) have shown an increase in plasma potassium concentration after a single dose of NaF. The rise in plasma potassium reported may reflect a toxic effect of sodium fluoride on the distal convoluted tubules, although other electrolytes(such as sodium) were not affected. Another possible explanation is the inhibition of (Na^+ - K^+)-activated ATPase, resulting in reduced uptake of potassium by cells (27). Alkaline phosphatases, are a group of enzymes found primarily the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). Small amounts are also produced by cells lining the intestines (isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules). Usually the total amount of alkaline phosphatases released from these tissues into the blood is measured. The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease. In the present study 20 and 30 mg/kg body weight of NaF caused a significant decrease in serum alkaline phosphatase while other doses caused no significant change in serum alkaline phosphatase. Since the total alkaline phosphate was measured, it is not clear as to which isoenzyme was exactly affected. However low serum alkaline phosphatase has also been shown to be associated with protein deficiency (11).

Collagen is broken down by collagenase enzyme into small fragments which are then broken down by proteolytic enzymes into amino acids. Since materials derived from the breakdown of collagen are removed by blood stream, the presence of Hyp fractions would reflect the collagen status of the body. In the present study though an initial dose of 10 mg/kg body weight NaF caused an increase in free Hyp all the higher doses caused a decrease in serum free Hyp. The pool of free Hyp has complex origin (1). It can arise from mature collagen, newly synthesized collagen, from dietary collagen or from propeptides of collagen. Therefore NaF may inhibit one or more of these processes to cause an decreased release of free Hyp in the serum. Our earlier (24) studies have shown that increased doses of mercuric chloride also caused a decrease in free serum hydroxyproline concentrations (24). Previous studies (16) have shown that the protein bound Hyp does not mirror collagen turnover but may be relevant to complement metabolism. Therefore a decrease in protein bound Hyp in serum after NaF treatment may indicate an altered complement metabolism. Higher doses of NaF caused a significant decrease in total Hyp concentration in the serum. Our previous studies have shown that different doses of NaF caused a significant decrease in total Hyp content of the kidneys (3) but an increase in total Hyp in lungs (unpublished data). Therefore the total Hyp in serum may reflect the the total Hyp and collagen status of the body. Sodium fluoride may cause decrease in collagen by promoting its breakdown in some tissues while it may also promote collagen formation in other tissues.

Acknowledgement - The authors would like to thank the Research Center, Center for Scientific and Medical Female Colleges, King Saud University, Riyadh for financial support to NJS and EAA. The authors would also like to thank King Abdul Aziz City for Science and Technology, Riyadh for financial support to EAA.

Other articles in this theme issue include references (31-46).

REFERENCES

1. Adams, E. and Frank, L., Metabolism of proline and the hydroxyprolines. *Annu. Rev. Biochem.* 1980, **49**: 1005-1061.
2. Ahmad, S., Hiyasat, A., Elbetieha, A. M. and Darmani, H., Reproductive toxic effects of ingestion of sodium fluoride in female rats. *Fluoride*. 2000, **33**: 279-284.
3. Al-Omireeni, E. A., Siddiqi, N. J. and Alhomida, A. S., Effect of different doses of sodium fluoride on various hydroxyproline fractions in rat kidneys. Kidney Research Journal. 2009, 1-7.
4. Bezerra de Menezes, L. M., Volpato, M. C., Rosalen, P. L. and Cury, J. A., Bone as a biomarker of acute fluoride toxicity. *Forensic. Sci. Int*. 2003, **137**, 209-214.
5. Birkner, E., Grucka-Mamczar, E., Machov, Z., Tarnawski R. and Polanaik Katowice, R. S., Disturbance of protein metabolism in rats after acute poisoning with sodium fluoride. *Fluoride*. 2000, **33**: 182-186.
6. Bouazia, H., Ketata, S., Jammoussi, K., Boudawara, T., Ayedi, F., Ellouze, F. and Zeghal, N., Effects of sodium fluoride on hepatic toxicity in adult mice and their suckling pups. *Pest. Biochem. Physiol*. 2006, **86**: 124 – 130.
7. Cicek, E., Aydin, G., Akdogan, M. and Okutan, H., Effects of chronic ingestion of sodium fluoride on myocardium in a second generation of rats. *Hum. Exp. Toxicol.* 2005, **24**: 79-87.
8. Dabek, A.W.D. and McKenzie, A. D., Survey of lead, cadmium, fluoride, nickel, and cobalt in food composites and estimation of dietary intakes of these elements by Canadians in 1986-1988. *J. AOAC. Int.* 1995, **78**: 897-909.
9. Friess, W., Collagen - biomaterial for drug delivery. *Eur. J. Pharmaceut. Biopharmaceut.* 1998, **45**: 113-136.
10. Gritsan, N. P., Miller, G. W. and Schumatkov, G. G., Correlation among heavy metals and fluoride in soil, air and plants in relation to environmental damage. *Fluoride*. 1995, **28**: 180- 188.

11. Lantz, O., Jouvin, M. H., De Vemejoul M. C. and Druet, P., Fluoride-induced chronic renal failure. *Am. J. Kidney Dis*. 1987, **10**: 136-139.
12. Lum, G., Significance of low serum alkaline phosphatase activity in a predominantly adult male population. *Clin. Chem.* 1995, **41**: 518-518.
13. Machoy – Mokrzynska A., Fluorine as a factor in premature aging. *Ann. Acad. Med. Stetin.* 2004, **50**: Suppl 1, 9 – 13.
14. Manna, P., Sinha, M. and Sil, P. C., A 43 kD protein isolated from the herb Cajanus indicus L attenuates sodium fluoride-induced hepatic and renal disorders in vivo. *J. Biochem. Mol. Biol*. 2007, **40**: 382-395.
15. Monsour, P.A., Kruger, B. and Smid, J. R., Effects of a Single Intravenous Dose of Sodium Fluoride on Plasma Electrolytes and Metabolites in Rats, Rabbits, and Cockerels. *J. Dent. Res.* 1985, 1281 – 1285.
16. Nagelschmidt, M. and Struck, H., Hydroxyproline fractions in serum and urine of rats during wound healing. *Res. Exp. Med*. 1977, **170**: 211-215.
17. Nemethy, G. and Scheraga, H. A., Stabilization of collagen fibrils by hydroxyproline. *Biochem*. 1986, **152**: 267- 273.
18. Neurath, C., Tooth decay trends for 12 year old in nonfluoridated and fluoridated countries. *Fluoride*. 2005, **38**: 324-325.
19. Pihlajaniemi, T., Myllya, R. and Krivirikko, K. I., Proly 4-hydroxylase and its role in collagen synthesis. *J. Hepatol*. 1991, **13**: S2-S7.
20. Qujeq, D., Laghaie, B., Gholipour, A., Solimani, N. and Hassenzadeh, S., Effects of sodium fluoride on total serum protein levels and transaminase activity in rats. *Biomed. Pharmacother*. 2002, **56**: 169 – 172.
21. Reddy, K. and Enwemeka, C. S., A simplified method for the analysis of hydroxyproline in biological tissues. *Clin. Biochem*. 1996, **29**: 225 – 229.
22. Shivarajashankara, Y. M., Shivashankara, A. R., Gopalakrishna Bhat, P. and Hanumanth Rao, S., Brain lipid peroxidation and antioxidant systems of young rats in chronic fluoride intoxication. *Fluoride*. 2002, **35**: 197-203.
23. Siddiqi, N. J., Al-Jafari, A. A. and Alhomida, A. S., Investigation of total, free, peptide-bound, protein-bound, soluble and insoluble collagen hydroxyproline content in tissues from the Arabian camel (*Camelus dromadarius*). *Cell. Biochem. Func.* 2000, **18**: 243-248.
24. Siddiqi, N.J. and Alhomida, A. S., Effect of mercuric chloride on various hydroxyproline fractions in rat serum. *Mol. Cell. Biochem*. 2005, **271**: 159-165.
25. Siddiqi, N.J. and Alhomida, A.S., Effect of mercuric chloride on urinary excretion of free hydroxyproline. *Med. Sci. Mon*. 2006, **12**: BR95-101.
26. Stannard, J.G., Skim, Y.S., Kirtsineli, M., Labropoulou, P. and Tsamtsouris, A., Fluoride Levels and Fluoride Contamination of Fruit Juices. *J. Clin. Pediatr. Dent*. 1991, **16**: 38-40.
27. Suketa, Y. and Terui, Y., Adrenal Function and Changes in Renal (Nat + K+)-ATPase Activity of Fluoride-intoxicated Rats. *Fluoride.* 1980, **13**:148-151.
28. Varghese, Z., Moorhead, J. F. and Wills, M. R., Plasma hydroxyproline fractions in patients with dialysis osteodystrophy. *Clin. Chim. Acta*. 1981, **110:** 105-111.
29. Xin, T.A.O., Zi Rong, X.U. and Wang, Y.Z., Effects of Dietary Fluoride Levels on Growth, Serum Indexes and Antioxidant Systems in Growing Pigs. *Turk. J. Vet. Anim. Sci*. 2006, **30**: 65-70.
30. Zhao, L. B., Liang, D. and Liang, W. W. L., Effects of a high fluoride water supply on children's intelligence. *Fluoride*. 1996, **29**: 190-192.
31. Tripathi S., Mahdi A. A., Hasan M., Mitra K. and Mahdi F., Protective potential of *Bacopa monniera (Brahmi)* extract on aluminum induced cerebellar toxicity and associated neuromuscular status in aged rats. *Cell. Mol. Biol.* 2011, **57 (1)**: 3-15.
32. Mishra A., Kumar S., Bhargava A., Sharma B. and Pandey A. K., Studies on *in vitro* antioxidant and antistaphylococcal activities of some important medicinal plants. *Cell. Mol. Biol.* 2011, **57 (1)**: 16-25.
33. Kumar A., Bhatnagar A., Gupta S., Khare S. and Suman, *sof* gene as a specific genetic marker for detection of *Streptococcus pyogenes* causing pharyngitis and rheumatic heart disease. *Cell. Mol. Biol.* 2011, **57 (1)**: 26-30.
34. Kumar Rai P., Kumar Rai D., Mehta S., Gupta R., Sharma B. and Watal G., Effect of *Trichosanthes dioica* on oxidative stress and CYP450 gene expression levels in experimentally induced diabetic rats. *Cell. Mol. Biol.* 2011, **57 (1)**: 31-39.
35. Kirby K.A., Singh K., Michailidis E., Marchand B., Kodama E.N., Ashida N., Mitsuya H., Parniak M.A., and Sarafianos S.G., The sugar ring conformation of 4'-ethynyl-2-fluoro-2'-deoxyadenosine and its recognition by the polymerase active site of hiv reverse transcriptase. *Cell. Mol. Biol.* 2011, **57 (1)**: 40-46.
36. Singh M.P., Pandey V.K., Srivastava A.K. and Viswakarma S.K., Biodegradation of Brassica haulms by white rot fungus *Pleurotus eryngii*. *Cell. Mol. Biol.* 2011, **57 (1)**: 47-55.
37. Baig M. S., Gangwar S. and Goyal N., Biochemical characterization of dipeptidylcarboxypeptidase of *Leishmania donovani. Cell. Mol. Biol.* 2011, **57 (1)**: 56-61.
38. Tripathi R., Gupta S., Rai S. and Mittal P. C., Effect of topical application of methylsulfonylmethane (msm), edta on pitting edema and oxidative stress in a double blind, placebo-controlled study. *Cell. Mol. Biol.* 2011, **57 (1)**: 62-69.
39. Bhatti G. K., Bhatti J. S., Kiran R. and Sandhir R., Biochemical and morphological perturbations in rat erythrocytes exposed to Ethion: protective effect of vitamin E. *Cell. Mol. Biol.* 2011, **57 (1)**: 70-79.
40. Chakravarty S. and Rizvi S. I., circadian modulation of Sodium-Potassium ATPase and Sodium - proton exchanger in human erythrocytes: *in vitro* effect of melatonin. *Cell. Mol. Biol.* 2011, **57 (1)**: 80-86.
41. Siddiqi N. J., Protective effect of Magnesium Chloride ON Sodium Fluoride induced alterations in various hydroxyproline fractions in rat lungs. *Cell. Mol. Biol.* 2011, **57 (1)**: 87-92.
42. Rohilla M. S., Reddy P. V. J., Sharma S. and Tiwari P. K., *In vitro* induction of the ubiquitous 60 and 70Kd heat shock proteins by pesticides monocrotophos and endosulphan in *Musca domestica*: potential biomarkers of toxicity. *Cell. Mol. Biol.* 2011, **57 (1)**: 100-111.
43. Janardhan Reddy P. V. and Tiwari P. K., Genomic structure and sequence analysis of *Lucilia cuprina* HSP90 gene. *Cell. Mol. Biol.* 2011, **57 (1)**: 112-121.
44. Mishra N. and Tewari R. R., Cytotoxic and genotoxic effects of mercury in house fly *Musca domestica* (Diptera: Muscidae). *Cell. Mol. Biol.* 2011, **57 (1)**: 122-128.
45. Tripathi M., Agrawal U. R. and Tewari R.R., Seasonal genetic variation in house fly populations, *Musca domestica* (Diptera: Muscidae). *Cell. Mol. Biol.* 2011, **57 (1)**: 129-134.

46. Rai D. K., Sharma R. K., Rai P. K., Watal G. and Sharma B., Role of aqueous extract of *Cynodon dactylon* in prevention of carbofuran- induced oxidative stress and acetylcholinesterase inhibition in rat brain. *Cell. Mol. Biol.* 2011, **57 (1)**: 135-142.

